BASOPHILIC AGGREGATION TEST
For Lead Absorption and Lead Poisoning

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OHIO DEPARTMENT OF HEALTH. Division of Adult Hygiene

R. H. MARKWITH, M. D.
Director of Health
Columbus, Ohio

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FOREWORD

The ultimate goal of the Ohio Department of Health is the prevention of occupational diseases by the control of those conditions in industry which affect the health of workers unfavorably.

The Industrial Hygiene program of the Ohio Department of Health is directed by the Adult Hygiene Division and this booklet has been assembled for the information of plant physicians and others responsible for the protection of the health of industrial workers.

THE BASOPHILIC AGGREGATION TEST FOR LEAD ABSORPTION AND LEAD POISONING

The need for a simple test for lead absorption and incipient lead poisoning has long been recognized. To make this test acceptable, it must fulfill several requirements. It must be a relatively simple procedure which every practitioner or technician can do in a few minutes. It must be as accurate as any accepted physiological determination and it must be approved by the test of time. The Adult Hygiene Division of the Ohio Department of Health employs the Basophilic Aggregation Test and believes it fulfills these requirements.

When the individual has absorbed lead in amounts greater than traces, one of the first body reactions is the formation of a larger than normal number of immature red cells and their appearance in the peripheral circulation. These immature blood cells may be distinguished from older red blood cells by several peculiar characteristics, the chief of which is the presence of basophilic material.

Attenion must be called here to the difference in the terms basophilic aggregation, basophilia, polychromatophilia, reticulocytes, and preformed stippled cells which exhibit punctuate basophilia. Basophilic aggregation is understood to mean a clumping of basophilic material into intracellular reticular forms so that a totality of all cells containing basophilic material may be determined. In the basophilic aggregation, the basophilic substances within the red cells may show fragmentation, combined reticulation and granulation, bands and wreaths at the periphery, coarse and fine stippling, balled or clumped basophilic material near the center of the cell, distended reticulum, and other forms. Hence, the number of basophilic aggregation cells seen in a smear stained by the technique subsequently described will be the total number of punctate stippled cells, reticulocytes, and all other cells which exhibit basophilic material.

PREPARATION OF THE SLIDES

It is important that slides of good quality be used and it is preferable to discard them (for basophilic aggregation tests) after they have been used once. These slides must be grease free as any great variance in the thickness and evenness of the smear will give erroneous results. The following procedure is recommended for cleaning slides:

(a) Place the slides in cleaning solution, consisting of concentrated sulphuric acid to which has been added a few crystals of potassium dichromate. It is recommended that the slides be allowed to remain in this solution for 3-4 days. (If time is not available the slides may be rinsed thoroughly in the warm cleaning solution).

(b) Rinse the slides thoroughly in tap water which is preferably hot.

(c) Transfer the slides to distilled water and then to 80% ethyl alcohol.

(d) Polish the slide with cheese cloth.

(e) Flame thoroughly and cool.

Note: The face and ends of the slide should not come into contact with grease from the fingers.

PREPARATION OF THE BLOOD SMEAR

A thin, even smear covering at least half of the length of the slide is desirable. Thinness should be such that 150 to 200 cells may be seen under the oil immersion objective. The following technique is to be employed:

(a) Obtain blood from finger or lobe of ear after cleaning part with alcohol and drying.

(b) Do not squeeze the blood out of the puncture.

(c) Do not use the first drop of blood.

(d) In order to obtain a thin smear, avoid taking too large a drop of blood.

(e) The drawing out or "pushing" of the blood droplet with the beveled edge of a second slide may be employed according to the technique to which one is accustomed.

DRYING OF THE BLOOD SMEAR

The proper drying of the blood smear is of utmost importance. According to Dr. C.P. McCord who first described this diagnostic test, "if the smear is permitted to become extensively dry, that is, longer than twelve hours, some of the basophilic containing cells will not lend themselves to aggregation of their basophilic material. On the other hand, insufficient drying facilitates removal of the cell during the staining period. Ordinarily, the optimum time lies between one to three hours". M.C. Hyler, however, has found that smears may be stained as late as sixty hours and accurate results obtained. It has been the experience of this Division that the optimum time for staining is from six to twelve hours.

FIXATION OF ONE-HALF OF THE SMEAR

After the smear has dried, one-half of the slide, on a longitudinal basis, is fixed. A faulty technique at this period may introduce sources of errors. Precaution must be taken that vapors from the alcohol used for fixing the slide do not partially fix the other half.

Several methods of fixing half of the slide may be employed. Dr. R. R. Jones of the U.S. Public Health Service prefers covering the longitudinal half of the slide with a strip of filter paper which is cautiously moistened with an absolute methyl alcohol (acetone free). An excess of alcohol is easily added and tends to run on the portion of the slide which is not to be fixed. Another method, the dipping of the longitudinal third of the slide was devised by M. C. Hyler. This is particularly useful when large numbers of slides are to be stained. A shallow utensil such as a petrie dish is filled with alcohol to such depth that one-third of the slide is submerged by dipping to the bottom of the utensil. After instantaneous dipping the slide is allowed to dry horizontally on muslin or gauze in such a fashion as not to permit any drainage on that portion of the slide for which fixation is wanted. This method is employed when large number of slides are to be stained. A third method of fixing is a modification of the Jones' method and consists of moistening the filter paper before it is placed on the slides, thus decreasing the danger of excess alcohol flowing to the unfixed portion. As soon as the filter paper is moistened, it is placed on the slide, covering a longitudinal half. The moistened filter paper is allowed to remain on the slide until it is dry. Dr. Vela-Gonzales of Mexico advises the Ohio Department of Health that he secures very good results by painting the longitudinal half of the slide by means of a camel's hair brush. This avoids an excess of methyl alcohol and one brush full is usually sufficient for three slides.

STAINING THE SMEAR

The slide is submerged for approximately 10 minutes in either Sussmann-Weindel or modified Manson stain. The time is not of great importance as a reliable stain may be obtained in as short a time as two minutes, and on the other hand, it is impossible to over-stain. The staining of the unfixed portion of the slide is in effect a laking process which removes the hemoglobin and clumps the basophilic material rendering it more visible under the microscope.

The formula is as follows:

Toluidine blue.....	0.5 gm.
Borax.....	0.05 gm.
Methylene blue (Loeffler's).....	5 cc.
Distilled water.....	100 cc.

The borax is added to the water which is heated. The toluidine blue is added and allowed to stand for a few minutes. Occasional stirring may hasten the solution. The methylene blue is then added. The solution is then filtered through a single No. 30 filter paper.

We have used the Sussmann-Weindel stain extensively, but have encountered difficulties in obtaining uniform toluidine blue. The modified Manson stain as used by the Ohio Department of Health is prepared as follows:

Sodium borate C.P.....	1.0 gm.
Methylene blue powder (Loeffler's)..	2.0 gm.
Distilled water	100.0 cc.

The water is brought to boiling and the sodium borate is added. After allowing the water to cool to room temperature, the methylene blue is added with vigorous stirring. The solution is then filtered. This stain is stable for at least two weeks. If it is kept in Coplin jars, the surface should be gently skimmed with a piece of filter paper before using.

Dr. Vela-Gonzales states that he has experienced considerable difficulty with the methylene blue and eliminates it entirely by using the following formula. It should be filtered before using.

Toluidine blue	2 gm.
Borax	2 gm.
Distilled water	100 cc.

BASOPHILIC AGGREGATION COUNTING

In counting the cells, an oil immersion objective and a 10 x ocular provided with a Whipple grid are used. The Whipple grid may be secured from any medical supply house at a nominal price. The outlines of the grid determine the microscopic field. The slide is first examined under low power to discover a uniform area in which the cells are well separated and of even distribution. Final examination is made with the oil immersion objective without the use of a cover slip. It will be noted that all the cells on the fixed portion of the slide are deeply stained, while on the laked portion of the slide, the normal erythrocytes appear as very faint shadows with indistinct cell outlines. The basophilic material in the red cells in the laked portion stands out sharply as coarse granules or as a reticulated network within the cell. Occasionally, in lightly stained cells, the eyes must be accomodated to the field before the cells can be seen.

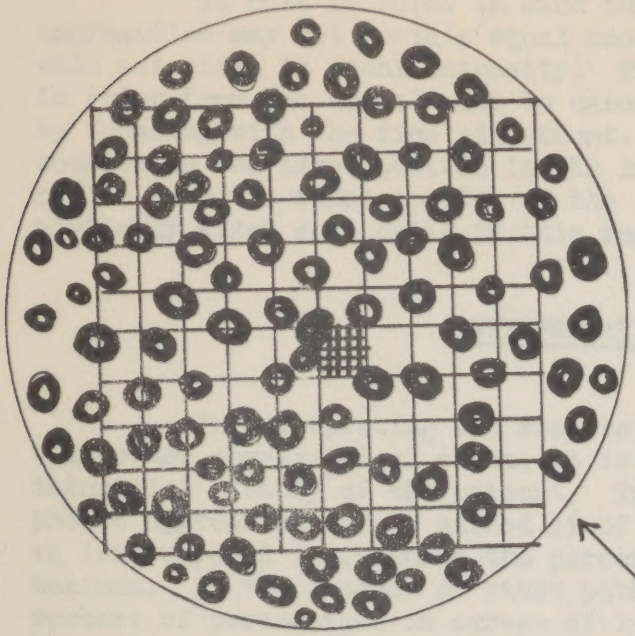
Determination of the percentage of basophilic aggregation cells is done in the following manner: A field is selected in an even portion of the slide on the fixed side. The total number of cells outlined by the grid in this field are counted. A mechanical counter facilitates the counting. The slide is then moved directly opposite to the unfixed laked half of the smear and the cells containing basophilic aggregation are counted in four contiguous fields. The slide is then moved opposite to the fixed side again and another field is counted. The procedure described in the preceding sentences is repeated until five fields have been counted on the fixed side and twenty fields on the laked side (see diagrams on the following page).

The results are expressed in percentage. Thus, if a total of 60 cells with basophilic aggregation was found in the 20 fields on the laked portion and 600 cells in the 5 fields on the fixed portion, the results would be 60 divided by 4 equal 15 divided by 600 or 2.5%. Thus, 2.5% of the total erythrocytes are basophilic aggregation cells.

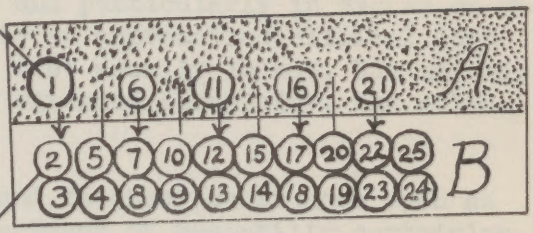
THE BASOPHILIC AGGREGATION TEST

FIGURE I

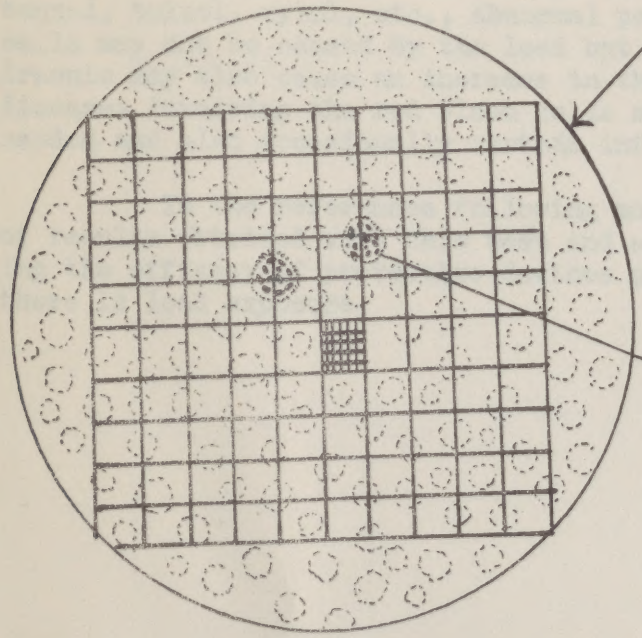
(Semi-schematic Drawings)



Section of fixed unlaked portion of smear. Determine total erythrocyte count and cellular abnormality. 10X Ocular and oil immersion. Straight lines are from Whipple Grid.



A. Fixed side (alcohol treated). Determine total cells per field.
B. Laked side. Determine basophilic cells per field.



Laked portion of blood smear showing two basophilic aggregation and faint outlines of non-basophilic containing erythrocytes. 10X Ocular and oil immersion.

Semi-schematic drawing of a typical basophilic aggregation containing cell. This is not a pre-formed punctate stippled cell.

It must be borne in mind that all the cells exhibiting basophilic aggregation may not contain equal amounts of basophilic material and hence will not stain in equal intensity. Therefore, any cells showing any variation in color from the normal must be carefully examined for basophilic aggregation by focusing with the fine adjustment. The basophilic cells should not be counted in the laked portion in the immediate vicinity of the line of separation from the fixed portion of the slide as alcohol may have flowed and partially fixed some cells in this region.

INTERPRETATION OF RESULTS

In interpreting the results of the Basophilic Aggregation test, as with other physiological tests, it is important to consider the medical and industrial history of the patient. In normal adults, cells containing basophilic aggregates rarely exceed 1% of the total number of orythrocytes, but in lead exposed individuals the percentages ordinarily lie above this normal maximum. In the absence of other pathology any finding in lead exposed workers of percentages in excess of 1-1/2% and particularly in excess of 2% suggests lead absorption and the possibility of approaching clinical lead poisoning. In chronic lead poisoning this test usually is not, but may be, positive. As lead poisoning progresses to extended chronicity, the reliability of the procedure diminishes.

In persons exposed both to lead and to other substances such as benzol, toluol, xylol, etc., abnormal percentages of basophilic containing cells may not be caused by the lead but by those other substances or by both. Arsenic may also cause an increase in these cells. Anemias and other types of diseases involving the red blood cells may cause the normal ranges to be exceeded and also occasionally certain infectious diseases.

In the references following may be found a more detailed description of results obtained with this test and also a discussion of its value in checking the efficacy of protective devices provided in manufacturing plants where there is lead exposure.

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